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ORIGINAL ARTICLE

Induction of shoot regeneration in cotyledon explants of the oilseed crop *Sesamum indicum* L.

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Abstract *Sesamum indicum* is an ancient oilseed crop known for its high quality edible oil and its medicinally important lignans. The crop is said to be recalcitrant to plant tissue culture thus limiting the use of modern biotechnology for its genetic improvement. We present here a protocol describing plant regeneration through adventitious shoot formation from cotyledons dissected from sesame seeds soaked for four hours in water. Subculturing of the cotyledons after two weeks of culture on to a fresh Murashige and Skoog medium leads to differentiation of adventitious shoots from the proximal cut end of the explant. Culture of cotyledons on a medium containing 9% sucrose for a couple of weeks prior to transfer to MS medium supplemented with 3% sucrose induced a higher frequency of shoot regeneration. The highest frequency of 25% adventitious shoot regeneration was observed for *S. indicum* variety UMA. This variety also turned out to be the best among the ten genotypes tested for shoot regeneration through tissue culture. While addition of IAA marginally improved regeneration, silver nitrate was found essential for enhancing the frequency of shoot regeneration. The regenerated shoots formed roots on full strength MS medium supplemented with 1 mg/l IBA and the rooted plants were established in soil.

1. Introduction

Sesamum indicum L. (syn *S. indicum* spp. *Orientale*) is an ancient oilseed crop known for its high quality edible oil and its varied uses in Ayurveda, Chinese and Tibetan traditional systems of medicines [4,5,19]. According to Index Kewensis *Sesamum,* of the family Pedaliaceae, is represented by 36 species distributed in East Africa and India [2,18]. Among these *S. indicum* is the only species that is cultivated. This species has a number of varieties cultivated in the USA, India, Russia, Kenya, China, and South Korea and to a lesser extent in Japan constituting a valuable sesame gene pool [14]. While 70% of the sesame seeds produced is used for oil extraction, the remaining is used in the manufacture of bakery and confectionery products [23]. Sesame seed yields 46–50% oil which is rich in mono and poly unsaturated fatty acids, 20–25% protein and various minerals [9]. Recently this crop has been receiving prominence due to its unique lignans such as sesamin, sesamolin, sesamol and tocopherols that have potential to cure hypertension, obesity and cancer. Even though sesame is in cultivation for a long time, the crop remains unattended

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due to low yields caused by biotic and abiotic stresses [19]. Lack of efficient tissue culture shoot regeneration system further limits genetic engineering of this crop for developing improved lines. Attempts have been made earlier to regenerate sesame in tissue cultures using explants such as shoot tips [11,15], nodes [10], cotyledons [26,28] and hypocotyls [3,31]. Significant success has been achieved for plant regeneration through somatic embryogenesis using hypocotyl or cotyledon as explants [13,16,31]. Excepting in rice, somatic embryogenesis has hardly met with success in genetic engineering of crop plants as compared to indirect *de novo* shoot regeneration from explants [20]. The shoot tips and nodes are unsuitable for *Agrobacterium* mediated transformation as they give multiple shoots from pre-existing primordial shoot and/or shoot buds [24]. Due to lack of a proper regeneration system, genetic engineering of the crop has yielded no tangible result so far [1,7,27,30]. Rather shoot regeneration reported in sesame so far is found to be unsuitable for transformation of any of the Indian varieties of sesame. It is known that the genotype of a crop strongly influences shoot regeneration efficiencies [16]. Therefore in this study we made an attempt to develop a protocol for shoot regeneration by evaluating the effect of genotypes on shoots regeneration from cotyledon thereby identifying the best variety of *S. indicum* for genetic engineering. To the best of our knowledge this is the first report on effect of genotype on shoot regeneration in Indian genotypes of sesame. The role of BAP, IAA and Silver Nitrate on shoot regeneration from the excised cotyledons of sesame is also reported here.

2. Materials and methods

2.1. Plant Materials

Sesamum indicum varieties used for screening in this study were TMV3, TMV4, TMV5, TMV6, VRI1, AKT64, TC25, RT127, Rajeshwari, and UMA. Seed samples of these varieties were procured from National Bureau of Plant Genetic Resources (NBPGR), New Delhi and maintained routinely in the experimental garden of our Department. These varieties differed in their seed color and the province for which they have been recommended for cultivation.

2.2. Surface sterilization of seeds and preparation of explant

About one hundred mature seeds of different accessions of sesame were washed thoroughly under running tap water. The seeds were then immersed in 50 ml of water containing 150 µl of Savlon, agitated for 10 min and then rinsed three times with distilled water. After removing the detergent, seeds were washed in water and immersed in 70% ethanol for 1 min followed by rinsing in sterile distilled water in a laminar airflow cabinet. Then the seeds were surface sterilized using sodium hypochlorite solution containing 2% chlorine for 5 min followed by a thorough wash in sterile distilled water for 3–4 times. The seeds were then left for soaking in sterile distilled water for 4 h. Aseptic seeds were then blotted on sterile Whatman No1 filter paper, and cotyledons devoid of embryonic axes were dissected from these sterilized seeds with a sterile blade under a biological microscope and transferred to the culture medium.

2.3. Culture medium and culture condition

In the present study MS basal medium is the one described in Murashige and Skoog [17]. pH of the medium was adjusted to 5.8 with 0.1 N NaOH or HCl before adding 0.8% agar powder for solidification, and then the medium was sterilized by autoclaving at 121 °C, 15 psi for 15 min. The medium was cooled to 40 degree Celsius before fortifying with filter sterilized plant growth regulators. All the cultures were maintained at 25 ± 1 °C and 16/8 h (light/dark) photoperiod provided by a two 40 µmol m^{-2} s^{-2} cool white fluorescent tubes.

2.4. Effect of genotype and high sucrose concentration on shoot regeneration

To test the effect of genotype and high sucrose concentration on shoot regeneration, cotyledons isolated from the seeds of different varieties of *S. indicum* were cultured on two sets of media. In the first set, the culture medium (Medium A), consisting of MS + 6.5 mg/l BAP + 1 mg/l IAA + 5 mg/l $AgNO_3$ supplemented with 3% sucrose was used. The hormone combination in the medium was in fact optimized and published in an earlier report for shoot regeneration in *S. indicum* variety VRI [7]. In the second set (Medium B) the cotyledons were initially cultured for two weeks on the medium of same composition but supplemented with 9% sucrose instead of 3% sucrose. After 2 weeks of culture, the explants from both the sets were shifted to MS medium of same composition but with 3% of sucrose. Periodic observations were made until 4 weeks of culture. By the end of fourth week a final observation was made, and the data were recorded to calculate the frequency and intensity of adventitious shoot formation from the cultured cotyledons.

2.5. Effect of BAP and IAA on cotyledons

The MS basal medium supplemented with different concentration of BAP (0, 4.5, 6.5, 8.5 and 10.5 mg/l) with $AgNO_3$ (5 mg/l) but with or without IAA (1 mg/l) was tested to find the effect of BAP alone or in combination with IAA on shoot regeneration in UMA variety of *Sesamum indicum* L. This experiment involved initial passage for two weeks on Medium B followed by transfer to Medium A as described in Section 2.4.

2.6. Effect of $AgNO_3$ on in vitro shoot regeneration

This experiment consisted of five treatments. MS medium supplemented with 6.5 mg/l BAP and 6.5 mg/l BAP + 1 mg/l IAA described in Section 2.5, was used as basal media to test the role of $AgNO_3$ (5 mg/l) on shoot regeneration from cotyledons. Medium devoid of hormones and supplements was used as control.

2.7. Rooting of shoots and hardening

The regenerated shoots formed in the above experiments were excised from surface of the explants and transferred to MS medium supplemented with 0, 0.1, 0.5, 1 mg/l IBA. The cultures were maintained as before and the data were recorded for obtaining the rooting efficiency. The rooted *in vitro* regen-